SR calcium release channel mechanisms for cardiac arrhythmias and their drug-based therapy

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The ryanodine receptors (RyR2) are the calcium release channels in sarcoplasmic reticulum (SR), which is the main Ca^{2+} store for cells of the heart. Mutations in RyR2 or calsequestrin cause arrhythmias as a result of increased diastolic Ca^{2+} release via RyR2. Such an abnormality manifests in Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), a form of cardiac arrhythmia that can lead to sudden death.

Therefore, a logical therapy for CPVT would be a RyR blocker to counter the mutation induced increase in RyR activity. However, it is not clear that inhibition *per se* has an anti-arrhythmic action because a commonly used RyR inhibitor, tetracaine, has a pro-arrhythmic action (Watanabe *et al.*, 2009). Recently, we found that flecainide, a Na^+ channel blocker of the Class I anti-arrhythmic group, was highly effective at preventing CPVT by a combined inhibition of the Na^+ channel and RyR2 (Watanabe *et al.*, 2009). Single channel recording of RyR2 in lipid bilayers showed that the kinetics of block by flecainide and tetracaine were very different. Flecainide decreased channel mean open time whereas tetracaine increased mean closed time (Hilliard *et al.*, 2010). In the same study, measurements of Ca^{2+} sparks revealed that flecainide decreased spark size but increased spark frequency resulting in zero net effect on diastolic SR Ca^{2+} leak and store load. On the other hand, tetracaine caused a decrease in spark frequency, resulting in an increased store load and increased spark size. The larger sparks were much more likely to initiate global Ca^{2+} transients, delayed after depolarisations in cardiomyocytes. However, it is not clear how the single channel blocking action of flecainide leads to its beneficial effect on Ca^{2+} spark properties. This is because our knowledge of the mechanism underlying Ca^{2+} sparks is incomplete. Here we present the first model to explain Ca^{2+} spark properties in terms of the structure of the diad (measured from electron microscopy) and the kinetics RyR2 gating determined from single channel recording. We use this model to show how the therapeutic action of flecainide on Ca^{2+} sparks relies on a drug that reduces RyR2 open times without affecting closed times.

In the model, the t-tubule is presented as a cylinder (125 nm radius and 3 μ m long). Wrapped around its circumference, at its midpoint, is the terminal SR, which is represented as a rectangular pancake (190 nm × 26 nm × tubule circumference). The terminal SR is connected to the longitudinal SR (LSR) at the centre of the pancake. LSR is represented by a labyrinth of tubules distributed throughout the cytoplasm. RyRs are located in the terminal SR membrane and release Ca^{2+} into the diad cleft (15 nm across) between the terminal SR and the t-tubule. In the present study, 16 RyRs were arranged in a 4×4 array with nearest neighbour separation of 31 nm. The kinetics of RyR opening was determined from single channel recording of luminal and cytoplasmic Ca^{2+} activation of RyR2 in bilayers, in the presence of Mg^{2+} (1 mmol/l), ATP (2 mmol/l). Ca^{2+} uptake was simulated by a well-established model for SERCa2 located in the LSR. Ca^{2+} diffusion and binding to Ca^{2+} indicators (Fluo3 and Fluo5), in and around the diad, were calculated by solving Ficks equation using Matlab on a MacBook Pro computer with quad i7 microprocessors.

Ca^{2+} sparks were triggered either by injecting Ca^{2+} into the diad cleft (*i.e.* simulating the opening of a single L-type Ca^{2+} channel) or by the opening of a single RyR by luminal Ca^{2+}. The Ca^{2+} released into the cleft by only two open RyRs was sufficient to trigger the remaining RyRs within 3 ms. Ca^{2+} release decreased the free [Ca^{2+}] in the terminal SR (from 1 mmol/l to 0.1 mmol/l within 5 ms), leading to a loss of Ca^{2+} release, depletion of Ca^{2+} in the cleft and a loss of RyR activation. All RyRs closed within 10 ms, the Ca^{2+} spark terminated in ~25 ms and [Ca^{2+}] in the terminal SR returned to 1 mmol/l with a time-constant of ~100 ms. The recovery time-constant was determined by the rate of Ca^{2+} diffusion from the LSR. Triggering of Ca^{2+} sparks during this restitution phase resulted in smaller Ca^{2+} sparks because of SR depletion. In our model simulations, a decrease in RyR mean open time from 4 to 2 ms, without increasing closed times, caused a 10% decrease is spark amplitude, a 40% increase in store load at maximal depletion and a 40% increase in the recovery of store load. The faster recovery explains the increased spark frequency caused by flecinaide.

Hilliard, F.A., D.S. Steele, D. Laver, Z. Yang, S.J. Le Marchand, N. Chopra, D.W. Piston, S. Huke, and B.C. Knollmann, (2010) Flecainide inhibits arrhythmogenic Ca^{2+} waves by open state block of ryanodine receptor Ca^{2+} release channels and reduction of Ca^{2+} spark mass. *Journal of Molecular and Cellular Cardiology* **48:** 293-301.

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